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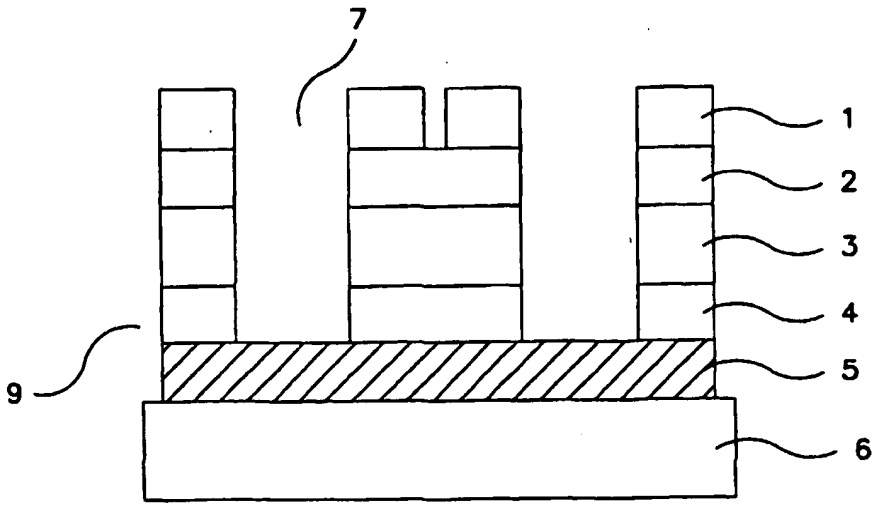
(54) Title: COLUMN AND ROW ADDRESSABLE HIGH DENSITY BIOCHIP ARRAY



(57) Abstract: The present invention provides a method and apparatus comprising a platform for a column-and-row-addressable high-density biochip array. The apparatus can be used as a high-density biochip array for electronic or electrochemical detection of molecular interactions between probe molecules bound to defined regions of the array and target molecules exposed to the array.

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COLUMN AND ROW ADDRESSABLE HIGH DENSITY BIOCHIP ARRAY

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the detection of biomolecules. Specifically, the invention relates to electronic or electrochemical detection of biomolecules using biochip arrays. In particular, the invention provides an apparatus comprising a platform for a column-and-row addressable, high-density, enhanced-sensitivity biochip array, and methods of use thereof.

2. Background of the Invention

A number of commonly utilized biological applications, including diagnoses of genetic disease, sequence-polymorphisms, analyses of gene expression, and studies of receptor-ligand interactions, rely on the ability to readily detect events related to probe-target interactions. In the past decades, autoradiography and fluorescence detection technologies have been used extensively in the molecular detection area.

The use of radioactivity to track molecules, however, presents serious health risks and requires adherence to burdensome regulatory procedures. Precautions must be taken by the user when using radiolabeled materials to avoid exposure to and contact with radioisotopes. Fluorescence technologies also require "labeling" to link the fluorescence marker to a biologically-relevant material, so that molecular interactions (such as nucleic acid hybridization or ligand/receptor binding) can be detected. Linkage of a fluorescent tag to a biomolecule inevitably increases the complexity of such molecules and can adversely affect probe/target interactions. In addition, fluorescence labeling is expensive, labor intensive and time consuming. This leads to increases in experimental cost (by requiring use of additional reagents, expensive hardware, and special equipment) and difficulty (resulting from the use of complicated procedures for handling and disposing of experimental byproducts). Furthermore, experimental reagents containing either radioactive or fluorescence tags often are of limited usefulness (for example, due to the radiochemical half-life of the radioisotope, or due to light sensitivity of the fluorescence label).

In contrast, electronic or electrochemical detection processes are based on

interactions between probe molecules on an electrode and target molecules in the detection solution that are detected as alterations in the electrical properties on the electrode. Electronic or electrochemical detection eliminates many of the disadvantages inherent in using radioactive or fluorescent labels to discern molecular interactions. More importantly, electronic or electrochemical detection devices can be made portable, as has been demonstrated in the case of widely available glucose sensors. Electrical and electrochemical detection devices thus provide an alternative molecular detection means that is safe, inexpensive, unobtrusive, and sensitive.

Electronic or electrochemical detection methods provide an attractive alternative to autoradiography or optical detection for identifying molecular interactions. In the prior art, electrochemical detection of biological molecules (hereinafter, "biomolecules") has generally been achieved by one of two methods. The first is selective modification at specific sites of a biomolecule (such as a nucleic acid or protein) with redox active moieties such as transition metal complexes. The second approach is intercalation of redox-active moieties, *e.g.* into duplex DNA strands. In addition, in the prior art most detection schemes have been carried out using either a single electrode or (at most) a few electrodes (typically, more electrodes were used for experimental redundancy, *i.e.*, in order to improve the accuracy of the result, rather than to increase experimental throughput).

Meade *et al.*, in U.S. Patent Nos. 5,591,578, 5,705,348, 5,780,234 and 5,770,369, disclosed methods of detecting a target sequence in a nucleic acid by hybridizing a target sequence with a single stranded nucleic acid that was modified with redox active moieties such as transition metal complexes.

International Patent Application, Publication No. WO 97/01646 teaches a method of detecting a nucleic acid by oxidizing at least one preselected base (*e.g.*, adenine or guanine) with a transition metal complex

A significant disadvantage of the electronic or electrochemical detection devices known in the prior art is that these devices use low-density arrays. For example, in U.S. Patent Nos. 5,670,322 and 5,532,128, Egger *et. al.* disclosed an apparatus for identifying biomolecular species within a sample substance using an array having a plurality of test sites upon which the sample was applied. Each test site had at least one electrode attached thereto for coupling with a second electrode surrounding the test site to form a capacitor in conjunction with the sample substance. Since the second electrode was preferably made

of a ring located outside the array and also acted to contain the sample solution, Egger's array required a large amount of sample solution (*i.e.*, enough to cover the area within the ring) in order for the array to function. More importantly, Egger's array could not be made row and column (x-y) addressable, limiting the density of the test sites in the array and thereby limiting the usefulness of this apparatus.

In U.S. Patent No. 5,653,939, Hollis *et al.* disclosed an x-y addressable array where test sites were composed of digitated electrodes located on a side bridge that was connected to both the x and y addressable conductive leads. However, the array of Hollis *et al.* is not practical to fabricate since the test sites are designed to bridge the x-and-y addressable conductive leads that are on two different planes with an insulating layer in-between.

Thus, there remains a need in this art for detecting molecular interactions by electronic or electrochemical means using high-density, row-and-column addressable arrays. In particular, the need is for an x-y addressable array that can be easily and cost-effectively fabricated, and that reduces the cost of performing various analyses, while increasing the effectiveness and utility thereof.

SUMMARY OF THE INVENTION

This invention provides an apparatus for electronic biomolecule detection using a column-and-row (x-y) addressable, high-density biochip array and methods of use thereof. Specifically, the apparatus facilitates electronic or electrochemical detection of molecular interactions between probe molecules bound to defined regions of a high-density addressable array and target molecules in a solution that is exposed to the array. The apparatus comprises a multiplicity of individual well structures, each said well further comprising two electrodes that can be individually addressed by applying an electric signal specifically to a particular address (well) in the array. In preferred embodiments, the bottom of the well comprises one electrode surface, while the second electrode surrounds the top of the well. Probe molecules include but are not limited to oligonucleotides, nucleic acids (DNA, RNA, etc), proteins, antibodies and peptides that are immobilized at a specific address comprising a well in the array.

Immobilization of such species is accomplished by direct anchoring of the probe molecules on the electrode surface, preferably by attaching the probe molecules onto a

supporting matrix on the surface of the electrodes. In the practice of the methods of the invention, the immobilized probe molecules are exposed to a solution containing an intended target molecule, for a time and under conditions sufficient for the probe molecules to bind to the target. An electrical signal is then applied to each of the individual well structures comprising the array. A change in the detected electrical signal in the presence of the solution (compared with the electrical signal detected in the absence of the solution) is used to determine whether a binding event between the probe and target has occurred at a particular address in the array.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a schematic representation of a cross-section view of the device platform.

Figure 2 illustrates a schematic representation of a top view of the device platform.

Figure 3 is a schematic diagram of the row/column configuration of a high-density array useful in the practice of the invention.

Figure 4 is a photograph of an x-y addressable array of the invention.

Figures 5A, 5B and 5C are masks for depositing electrode and insulating layers in the x-y addressable arrays of the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides an apparatus for electronic or electrochemical detection of biomolecules. For the purposes of this invention, the term "biomolecule" is intended to encompass biologically-derived molecules that interact specifically with one another. Non-limiting examples of such biomolecules are complementary nucleic acid strands, ligand/receptor, agonist/receptor and antagonist/receptor pairs, antigens and their cognate antibodies, enzyme/substrate and enzyme/inhibitor combinations. In general, the

biomolecules of the invention comprise a binding pair, whereby there is a specific interaction between each member of the pair. As used herein, one member of the pair is conveniently termed a "target" and the other a "probe." As used herein, "probe" molecules are preferably bound to a solid substrate and "target" molecules comprise a sample to be tested for the presence, amount or concentration of the "target." Target molecules can be any of these biomolecules, most preferably wherein at least one of the target molecules specifically interacts with one of the probe molecules.

In preferred embodiments, the probe molecules are oligonucleotides. Oligonucleotide probes of length 5 to 1000 basepairs (bp), more preferably 5 to 100bp and most preferably about 5 to 40bp, can be attached to the attachment medium. Targets include PCR amplicons, genomic DNA, cDNA and synthetic and cellular RNA. For protein binding devices, probes can be oligonucleotides such as aptamers or other oligonucleotides having well-defined secondary structure that will bind to proteins. Alternatively, peptides, antibodies or antigens can be immobilized to perform binding assays.

The present invention provides an apparatus for electronic or electrochemical detection of biomolecules using a row-and-column ("x-y") addressable array having a plurality of addressable sites to which a target sample is applied, and methods of use thereof. Each addressable site comprises at least two electrodes that are connected to two conductive lead lines that can be addressed in a x-y coordination fashion. The addressable site is preferably a well structure as defined herein wherein the bottom of the well comprises the surface of one electrode, and the top of the well comprises the second electrode. In alternative embodiments, each said well structure further comprises at least one additional electrode, preferably a reference electrode, positioned between the top and bottom of the well. In more preferred embodiments, the devices of the invention comprise at least two electrodes, and a multiplicity of probe molecules immobilized in proximity to the electrodes, wherein the probe molecules are preferably immobilized at the surface of at least one of the electrodes.

Device embodiments of the invention are useful for performing methods for biomolecule detection by either electrochemical or electronic means. As used herein, the term "electrochemical detection" is intended to encompass methods based on oxidation/reduction (redox) processes induced by electron transfer between electrodes, most preferably mediated by an electrochemical reporter group attached to the probe

moiety, the target moiety, or both. As used herein, the term "electronic detection" is intended to encompass methods that rely on impedance changes (such as resistance, capacitance and inductance) due to differences in electronic state occupancy in the biomolecules in the bound and unbound conformations.

An additional advantage of the devices of the invention is that both impedance and electrochemical measurements can be performed in the same assay using the same x-y addressable array to enhance the sensitivity and reduce system "noise" resulting from nonspecific binding of biomolecules. For example, in probe arrays comprising nucleic acids, it is generally not possible to perform electrochemistry on the probe molecules themselves, since they cannot participate in redox reactions under readily-achievable voltage potentials unless they are linked to an electrochemical reporter group that can participate in such a redox reaction. However, an impedance measurement of the probe array can be performed in either the presence or absence of such electrochemical reporter groups to monitor the quality of probe attachment at each particular address prior to introduction of the target. This permits the user to set a reliable baseline for each x-y addressable position (or "pixel") in the array prior to performing an assay. For electrochemical detection of nucleic acid hybridization of targets to oligonucleotide probes in a high-density, x-y addressable array, application of a low electric field (< 150 mV, less than the redox potential of most electrochemical reporter groups) can be used to concentrate target nucleic acid molecules in proximity of the array. This significantly enhances detection sensitivity and reduces probe-target interaction time; as a consequence, assay time is also reduced. After such electrically-enhanced hybridization is performed, electrochemistry can be performed on the molecular complex at or near the redox potential of the electrochemical reporter group where molecules tagged with an electrochemical reporter groups have hybridized to the immobilized probe. This provides an additive signal to be measured that distinguishes background binding from specific binding at each address in the x-y addressable array. This feature of the assay provides an increased assay sensitivity by reducing the baseline (noise or background) signal due to non-specific binding of the target to the probe. This feature is also a unique characteristic of the multielectrode device structure described here and is not found in the prior art.

In preferred embodiments, the electrochemical reporter groups comprise a transition metal complex, most preferably containing a transition metal ion that is ruthenium, cobalt, iron or osmium.

The preferred embodiment of the present invention and its advantages over previously investigated electronic or electrochemical detection devices are best understood by referring to Figures 1 and 2 and Example 1. Like numerals have been used in the drawings for like and corresponding parts.

Figure 1 illustrates a schematic representation of a cross-section view of the device platform **9** of the present invention. The device **9** is built on a solid supporting substrate **6**, which can be made of any solid, non-porous substance, including and preferably glass, plastic, ceramic, semiconductor or printed circuit board (PCB). Patterned conductive electrodes **5** are fabricated on top of the solid supporting substrate. The patterned conductive electrodes **5** are fabricated of electrically-conductive metals (including but not limited to transition metals such as aluminum, gold, copper, silver, platinum, chromium, and titanium), transparent conductors (such as indium-tin-oxide and zinc oxide), conductive plastics (such as polymers like polythiophenes, polyanilines, polypyrroles, and metal impregnated polymers), or conductive carbon (such as graphite).

The devices are advantageously formed by standard fabrication techniques used in semiconductor manufacturing. Non-limiting examples of methods for producing solid substrates comprising the device platforms of the invention include but are not limited to thermal evaporation, wire bonding, metallization (evaporation, plating, sputtering over a shadow mask), dielectric deposition (by plasma, chemical vapor deposition or sputtering), wet or dry chemical etching, reactive ion etching, or liftoff after the desired pattern has been defined using conventional photolithography.

A layer of insulative dielectric material **4**, which can be made of polymers, metal oxide or nitrides such as SiO_2, SiN_x or AlO_x is placed on top of the patterned conductive electrodes **5**. An optional layer of conductive metal **3** is placed over the insulative dielectric material **4**. This layer constitutes a reference electrode. In a preferred embodiment, the conductive metal layer **3** is silver, which is then advantageously converted to silver /silver chloride at a later stage in manufacturing. A second layer of insulative dielectric material **2** is then placed on top of the conductive electrode layer **3**. In embodiments not comprising a reference electrode **3**, a continuous dielectric layer **2** comprising layers **2** and **4** as set forth herein are deposited. The second layer of insulative dielectric material **2** is optionally made of the same materials as the insulative layer **4**.

Patterned conductive electrodes **1** constructed on top of the second layer of insulative dielectric material **2** constitute the final layer of each addressable site in the

device **9**. Well structures **7** are fabricated from this device by conventional photolithography or laser drilling methods used in the semiconductor industry for PCB manufacturing. These wells can have rectangular, circular, trapezoidal or other polygonal openings. Additionally, the well walls may be either straight or curved, and may have an arbitrary angle with respect to the bottom electrode **5**. An optional center electrode can alternatively protrude into the well area, as shown in Figure 3.

Figure 2 illustrates a schematic representation of a top view of the apparatus of the invention. The conductive electrodes **1** are preferred to be oriented in a direction orthogonal to the patterned conductive electrodes **5**, generating row (*i.e.*, patterned electrodes **5**) and column (*i.e.*, conductive electrodes **1**) addressable high-density electronic or electrochemical mini-cells (*i.e.*, well structures **7**) with optional reference electrodes built in-between. The well structure is preferably produced wherein the bottom of the well structure comprises the top of electrode **5** surface, while the top of the well structure is surrounded by the second electrode **1**.

The proposed device **9** can be used as an x-y addressable, high-density biochip array when biological probes **10** are immobilized on the patterned electrodes **5** inside each well structure **7**. The apparatus is capable of detecting changes in the electrical properties of the probes **10** in each well structure arising from the interaction of the probes **10** with target molecules **11**. Though the inventive apparatus is useful for single species detection, where only a few test wells (low density) are required, the advantages of the invention are more pronounced in a high density array where hundreds, thousands, or millions of test wells are integrated in one array.

In a preferred embodiment, the probe molecules may be oligonucleotides, nucleic acids (such as DNA or RNA), proteins, peptides, antibodies or small molecules such as ligands, wherein probe molecules are chemically modified to contain anchoring groups that permit immobilization. Preferred modifications to the oligonucleotides useful in the practice of the invention include but are not limited to -OH, $-NH_2$, -SH, -COOR (where R = H, lower (C_{1-12}) alkyl, aryl, heterocyclic alkyl or aryl, or a metal ion), -CN, or -CHO. Immobilization of such derivatized probes is accomplished by direct attaching of the probe molecules on the electrode surface through a functional group such -OH, -SH, $-NH_2$.

Alternatively, probe molecules can be efficiently immobilized on the electrode surface through an intermediate species, termed a "spacer." In these embodiments, the surface of the electrode **5** is first modified with an intermediate species that carries

functional groups such as hydroxyl (-OH), amino ($-NH_2$), thiol (-SH), carboxyl ester (-COOR, where R = H, lower (C_{1-12}) alkyl, aryl, heterocyclic alkyl or aryl, or a metal ion), nitrile (-CN), or aldehylde (-CHO), which can react with the probe molecules functionalized with complementary members of the aforementioned anchoring groups.

In another embodiment, the surface of the electrodes **5** is covered with a layer of polymer matrix. In these embodiments, probe molecules are attached onto a supporting matrix on the surface of the electrodes using the functional chemistry mentioned above. The polymer matrix is preferably selected to be polypyrrole, polythiophene, polyaniline, polyacrylamide, agarose gel, polyethylene glycol, cellular, sol gels, dendrimers, metallic nanoparticles, carbon nanotubes, and their copolymers. To increase the probe loading capacity, porous matrix such as polyacrylamide, agarose, or sol gels are preferred.

Electronic or electrochemical detection of molecular interactions between probe and target molecules is achieved by devices having the structure, *for example*, as depicted in Figure 1. The electric or/and electrochemical methods used to interrogating the biomolecule targets may be selected from, but are not limited to, AC impedance, cyclic voltammetry (CV), pulse voltammetry, square wave voltammetry, AC voltammetry (ACV), hydrodynamic modulation voltammetry, potential step method, potentiometric measurements, amperometric measurements, current step method, and combinations thereof.

In an alternative embodiment, an active driving circuit such as the one used in an active matrix liquid crystal display device can be built underneath or nearby each test well site to replace the electronic column and row drivers for x-y addressing such as the one used in the passive matrix liquid crystal display device.

In the practice of the invention, a high-density, x-y addressable probe array is exposed to an electrolyte solution containing a target molecule for a time and under conditions sufficient for the target to bind to a probe present in at least one of the particular addresses of the column-and-row addressable array. A voltage potential or other electric signal is applied to the each of the electrodes comprising each of the addressable sites through the x-y addressable column and row electrodes. Changes in the electrical properties or electrical signals from a particular electrode at a particular site in the x-y addressable array arising from interactions between probe molecules on the electrode and target molecules in the solution are detected to determine the presence and concentration of the target molecules in the solution.

In certain advantageous embodiments, electrical cross-talk between electrodes is reduced or eliminated in the x-y addressable array during target interrogation with an external electrical source. In these embodiments, the electrodes at the top of the wells are covered with an array of microfluidic channels. These channels are designed to be independently isolated from each other, with each having its own isolatable liquid inlet and outlet port. In addition to functioning as an electrical isolator, the channels also act as containers or reaction chambers for liquid during probe-target hybridization, enzymatic reactions and target interrogation with the external electrical source. In an active driving array where the x-y addressable columns and rows are replaced by an active driving circuit built underneath or nearby each test well site, the microfluidic channels can be replaced by a single chamber that covers all the test sites with

Electrolyte solutions useful in the apparatus and methods of the invention include any electrolyte solution at physiologically-relevant ionic strength (equivalent to about 0.15M NaCl) and neutral pH. Nonlimiting examples of electrolyte solutions useful with the apparatus and methods of the invention include but are not limited to phosphate buffered saline, HEPES buffered solutions, and sodium bicarbonate buffered solutions. In alternative embodiments useful for electrical detection methods provided by the invention, the electrolyte solution comprises metal cations or polymerized cations that are ion conductive and capable of reacting with probes or probe-target complexes.

The Examples, which follow, are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

EXAMPLE 1

Fabrication of a linear microarray with four wells

A linear test microarray with four wells was fabricated on a 3" inch silicon wafer as follows. A photograph of the array is shown in Figure 4.

The linear test array was fabricated by conventional photolithography in a class 100 clean room and fabrication was performed using three layers of masks as shown in Masks 12 (Figure 5A), 14 (Figure 5B) and 16 (Figure 5C).

A three inch silicon wafer was cleaned using a solution of $NH_4OH:H_2O$ (1:10 v/v), rinsed with de-ionized water, and then dried using a stream of nitrogen at room temperature. On the top of the wafer, 2000Å SiO_2 was deposited by conventional

chemical vapor deposition technique.

The array was then prepared sequentially as follows.

1. Bottom Electrode Formation

After cleaning the SiO_2-prepared substrate using a solution of $NH_4OH:H_2O$ (1:10 v/v), a de-ionized water rinse, and drying with a stream of nitrogen as described above, a thick (5 micron) photoresist (PR) layer was spin-coated on the wafer through a three stage process of spin-coating and softbaking. Using **Mask 12** (shown in Figure 5A) to protect the portion of the substrate that forms the bottom electrode, the surface was exposed to an ultraviolet light source using a wavelength of 365 nm and an intensity of 6 mW/cm^3. Following this treatment, the PR was hardbaked and developed. After removal of **Mask 12**, the following metals were deposited sequentially by evaporation: Ti (to a thickness of 1.0 Angstrom), Au (to a thickness of 21,000 Angstrom), and Ti (to a thickness of 500 Angstrom). After evaporative deposition of these metal layers, a liftoff protocol was used to produce the bottom patterned electrode.

2. Top Electrode Formation

After cleaning the bottom electrode-prepared substrate using a solution of $NH_4OH:H_2O$ (1:10 v/v), a de-ionized water rinse, and drying with a stream of nitrogen as described above, the wafer was coated with a thick (8 micron) layer of PR, as described above. Using **Mask 14** (Figure 5B) to protect the portion of the substrate that forms the top electrode, the surface was exposed to an ultraviolet light source using a wavelength of 365 nm and an intensity of 6 mW/cm^3. Following this treatment, the PR was hardbaked and developed as described above After removal of **Mask 14**, the following metals were deposited sequentially by evaporation: Ti (to a thickness of 1.0 Angstrom) and Au (to a thickness of 21,000 Angstrom). After evaporative deposition of these metal layers, a liftoff protocol was used to produce the top patterned electrode, as described above.

3. Well Structure Formation

After cleaning the top electrode-prepared substrate using a solution of $NH_4OH:H_2O$ (1:10 v/v), a de-ionized water rinse, and drying with a stream of nitrogen as described above, the wafer was then coated with a 4 micron layer of PR. The surface was exposed to an ultraviolet light source using a wavelength of 365 nm and an intensity of 6

mW/cm^3. Following this treatment, the PR was hardbaked and developed as described above. The wafer was then subjected to buffer oxide etching solution (4:1) until each well opening was cleared. The PR was removed by placing in a Branson 4000 Sonicator.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

WHAT WE CLAIM IS:

1. An apparatus for electronic or electrochemical detection of molecular interactions between an immobilized probe and a target molecule, comprising:

(a) a solid, non-porous supporting substrate,

(b) a first conductive electrode layer placed on top of the supporting substrate,

(c) a first insulative dielectric layer placed on top of the first conductive electrode layer,

(d) a first conductive metal layer placed on top of the first insulative dielectric layer,

(e) a second insulative dielectric layer placed on top of the first conductive metal layer,

(f) a second conductive electrode layer placed on top of the second insulative dielectric layer and oriented orthogonally to the first conductive electrode layer, and

(g) a plurality of well structures having walls formed by the absence of regions of the second conductive electrode layer, second insulative dielectric layer, first conductive metal layer, and first insulative dielectric layer, wherein the bottoms of said wells are formed by the first conductive electrode layer, and wherein the wells have probes immobilized thereon.

2. The apparatus of claim 1, wherein the supporting substrate comprises glass, plastic, ceramic, silicon, or printed circuit board.

3. The apparatus of claim 1, wherein the first conductive electrode layer comprises a metal selected from the group consisting of aluminum, gold, copper, silver, platinum, chromium and titanium.

4. The apparatus of claim 1, wherein the first conductive electrode layer comprises a transparent conductor.

5. The apparatus of claim 1, wherein the first conductive electrode layer comprises a conductive plastic or conductive carbon.

6. The apparatus of claim 1, wherein the first insulative dielectric layer

comprises a polymer, metal oxide or nitride.

7. The apparatus of claim 1, wherein the second insulative dielectric layer comprises a polymer, metal oxide or nitride.

8. The apparatus of claim 1, wherein the second conductive electrode layer comprises a metal selected from the group consisting of aluminum, gold, copper, silver, platinum, chromium and titanium.

9. The apparatus of claim 1, further comprising a reference electrode comprising a conductive electrode layer.

10. The apparatus of claim 1, wherein the conductive electrode layer of the reference electrode comprises a metal selected from the group consisting of aluminum, gold, copper, silver, platinum, chromium and titanium.

11. The apparatus of claim 1, wherein the conductive electrode layer of the reference electrode comprises silver or a mixture of metallic silver and silver chloride.

12. The apparatus of claim 1, wherein the probes are nucleic acids.

13. The apparatus of claim 12, wherein the probes are oligonucleotide probes.

14. The apparatus of claim 1, wherein the probes are peptides.

15. The apparatus of claim 12, 13 or 14, wherein the probes are covalently attached to the first conductive electrode.

16. The apparatus of claim 1, wherein the well structures are fabricated by photolithography or laser drilling.

17. The apparatus of claim 15, wherein the probes are attached to the first conductive electrode through a spacer or a polymer matrix.

18. An apparatus for electronic or electrochemical detection of molecular interactions between an immobilized probe and a target molecule, comprising:

(a) a solid, non-porous supporting substrate,

(b) a first conductive electrode layer placed on top of the supporting substrate,

(c) a first insulative dielectric layer placed on top of the first conductive electrode layer,

(d) a second conductive electrode layer placed on top of the second insulative dielectric layer and oriented orthogonally to the first conductive electrode layer, and

(e) a plurality of well structures having walls formed by the absence of regions of the second conductive electrode layer, first insulative dielectric layer wherein the bottoms of said wells are formed by the first conductive electrode layer, and wherein the wells have probes immobilized therein.

19. The apparatus of claim 18, wherein the supporting substrate comprises glass, plastic, ceramic, silicon, or printed circuit board.

20. The apparatus of claim 18, wherein the first conductive electrode layer comprises a metal selected from the group consisting of aluminum, gold, copper, silver, platinum, chromium and titanium.

21. The apparatus of claim 18, wherein the first conductive electrode layer comprises a transparent conductor.

22. The apparatus of claim 18, wherein the first conductive electrode layer comprises a conductive plastic or conductive carbon.

23. The apparatus of claim 18, wherein the first insulative dielectric layer comprises a polymer, metal oxide or nitride.

24. The apparatus of claim 18, wherein the second conductive electrode layer comprises a metal selected from the group consisting of aluminum, gold, copper, silver, platinum, chromium and titanium.

24. The apparatus of claim 18, wherein the probes are nucleic acids.

25. The apparatus of claim 18, wherein the probes are oligonucleotide probes.

26. The apparatus of claim 18, wherein the probes are peptides.

27. The apparatus of claim 24, 25 or 26, wherein the probes are covalently attached to the first conductive electrode.

28. The apparatus of claim 18, wherein the well structures are fabricated by photolithography or laser drilling.

29. The apparatus of claim 27, wherein the probes are attached to the first conductive electrode through a spacer or a polymer matrix.

30. A method for electric or electrochemical detection of molecular interactions between an immobilized probe and an electrochemically active reporter-labeled target molecule, comprising:

(a) exposing a plurality of well structures in an x-y addressable array to an electrolyte solution containing an electrochemically-labeled target molecule in order to generate probe-target complexes

(b) detecting an electrical signal in at least one of the plurality of well structures in

the x-y addressable array to which probes have been attached.

31. The method of Claim 30, wherein molecular interactions between an immobilized probe and an electrochemically active reporter-labeled target molecule are detected by AC impedance, cyclic voltammetry, stripping voltammetry, pulse voltammetry, square wave voltammetry, AC voltammetry, hydrodynamic modulation voltammetry, potential step method, potentiometric measurements, amperometric measurements, current step method, or combinations thereof.

32. The method of Claim 30, wherein molecular interactions between an immobilized probe and an electrochemically active reporter-labeled target molecule are detected by AC impedance measured over a range of frequencies before and after exposing the plurality of well structures in the x-y addressable array to an electrolyte solution containing the electrochemically active reporter-labeled target molecule.

33. The method of Claim 30, wherein molecular interactions between an immobilized probe and an electrochemically active reporter-labeled target molecule are detected by AC impedance measured by transient methods with AC signal perturbation superimposed upon a DC potential applied to an electrochemical cell.

34. The method of Claim 30, wherein molecular interactions between an immobilized probe and an electrochemically active reporter-labeled target molecule are detected by AC impedance measured by impedance analyzer, lock-in amplifier, AC bridge, AC voltammetry, or combinations thereof.

35. The method of Claim 30, wherein the molecular interactions detected thereby are single base mismatches within nucleic acid probe-target complexes.

36. The method of Claim 30, wherein the molecular interaction detected is quantification of electrochemically active reporter-labeled target molecules in a reaction mixture for gene expression analyses.

37. The method of Claim 30, wherein the electrochemically active reporter-labeled target molecules are labeled with electrochemical reporter groups comprising a transition metal complex.

38. The method of Claim 37, wherein the transition metal ion is ruthenium, cobalt, iron, or osmium.

39. A method for electrical detection of molecular interactions between an immobilized probe and a target molecule, comprising:

(a) contacting a plurality of well structures in an x-y addressable array to

which probes have been attached with an electrolyte solution,

(b) measuring the impedance in the well structures,

(c) exposing the well structures to an electrolyte solution containing a target molecule

to generate probe-target complexes,

(d) measuring the impedance at the well structures, and

(e) detecting molecular interactions between the immobilized probe and the target

molecule by detecting a change in the measured impedance in the well structures

before and after exposing the probes in the well structures to the electrolyte solution containing the target molecule.

40. The method of Claim 39, wherein the electrolyte solution comprises metal, non-metal or polymerized cations that are ion-conductive and capable of reacting with probes or probe-target complexes.

41. The method of Claim 39, wherein impedance is measured over a range of frequencies prior to and after exposing the well structures to a reaction mixture containing the target molecule.

42. The method of Claim 39, wherein impedance is measured by transient methods with AC signal perturbation superimposed upon a DC potential applied to an electrochemical cell.

43. The method of Claim 39, wherein impedance is measured by impedance analyzer, lock-in amplifier, AC bridge, AC voltammetry, or combinations thereof.

43. The method of Claim 39, wherein the molecular interactions detected thereby are single base mismatches within nucleic acid probe-target complexes.

45. The method of Claim 39, wherein the molecular interaction detected is quantification of target molecules in a reaction mixture for gene expression analyses.

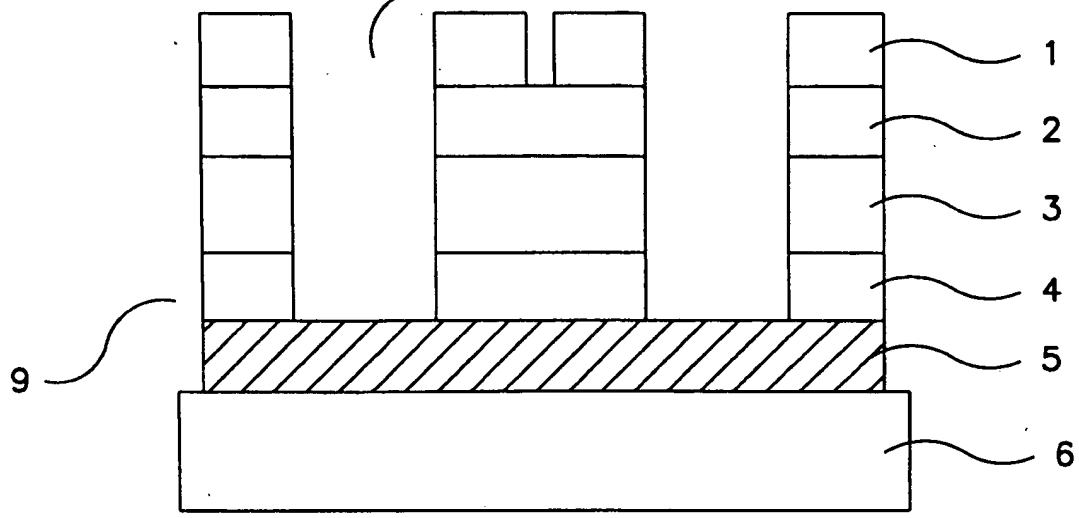

FIG. 1

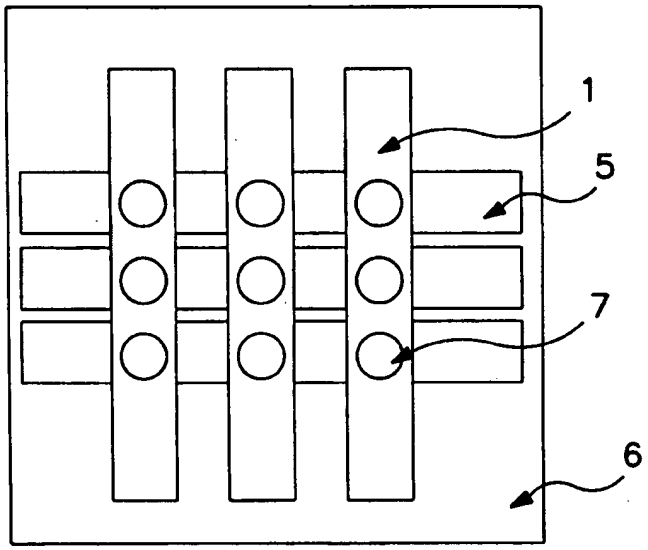

FIG. 2

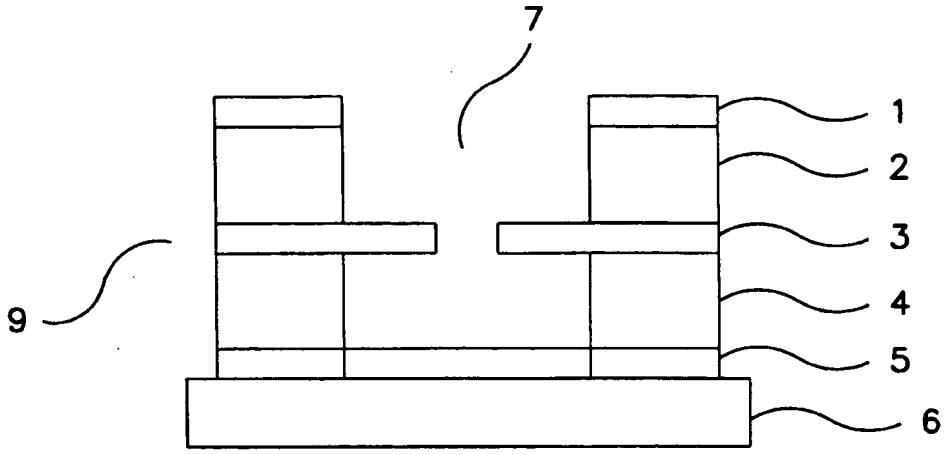

FIG. 3

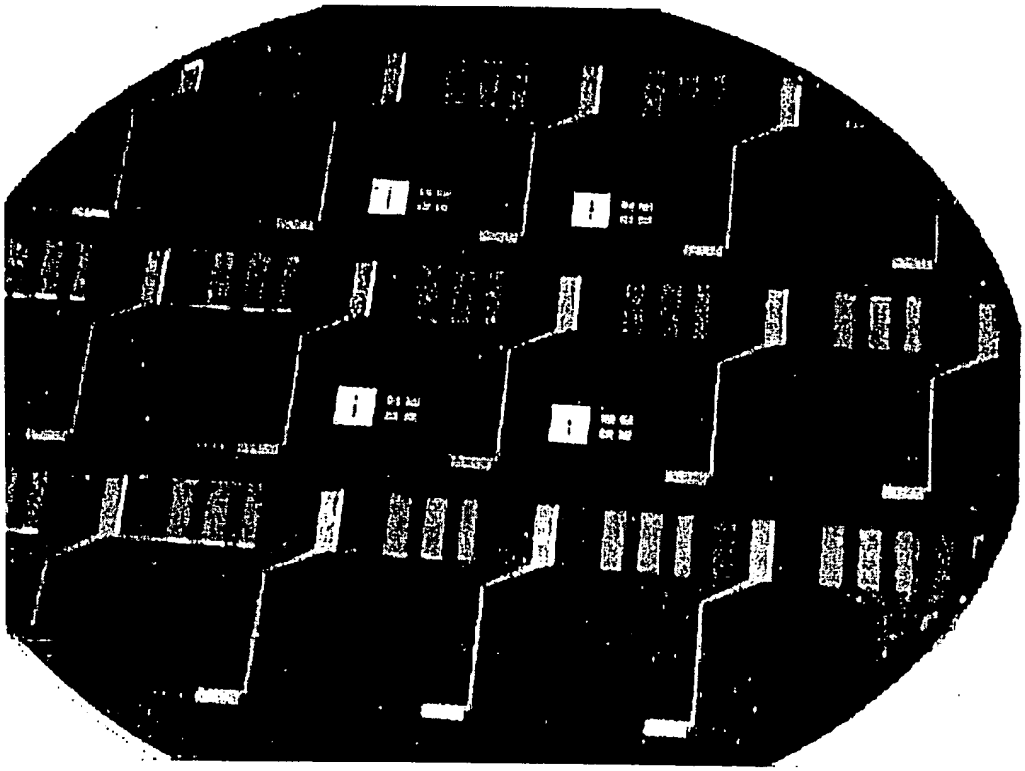

FIG. 4

FIG. 5A

Mask 12

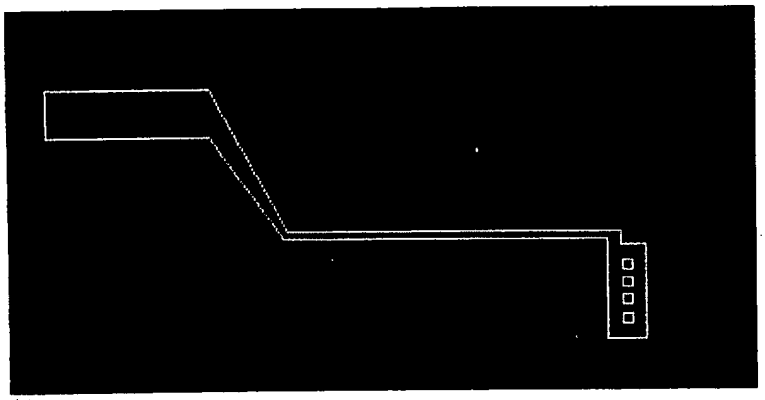

FIG. 5B

Mask 14

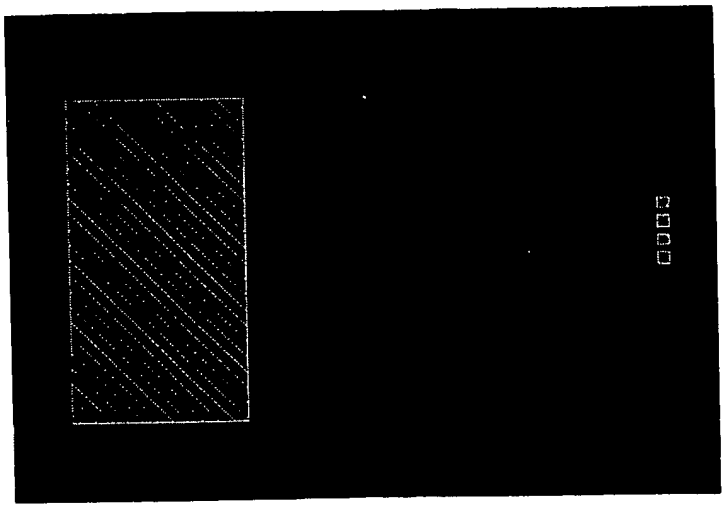

FIG. 5C

Mask 16